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Predation on two species of surface dwelling Collembola
A study with radio-isotope labelled prey

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With one figure

1. Introduction

The position of springtails in the foodweb of terrestrial communities makes it likely that predation plays an important part in the regulation of their numbers. Experiments carried out by Sheals (1955) and Edwards (1969) seem to confirm this assumption. They treated woodland soil with insecticides which killed the mite predators to a larger extent than the springtails. The density of the springtails increased markedly following the suppression of the mite predators.

The experiments described in this paper were designed to evaluate the importance of predation as a mortality factor for the populations of two species of surface dwelling Collembola. This includes an examination of the stability of this mortality factor, for which it was necessary to know the number of predatory species and individuals.

The species of springtails studied were *Orchesella cincta* (Linné) and *Tomocerus minor* (Lubbock). These species clearly differ in ecological aspects. Joosse (1970) showed that *T. minor* has to live within a narrow range of high humidities, while *O. cincta* tolerates a broader range. Related to this difference *T. minor* showed a higher degree of aggregation in humid places and a lower locomotory activity than *O. cincta*. This points to the probability that *T. minor* is more limited in its habitat exploitation than *O. cincta*, which may lead to a different predation chance.

Predation on springtails occurs unseen in the litter layer. As a method adapted to this situation, the radioactive marking of the prey with P^{32} was chosen. The possible harmful effects of radioactive radiation on living matter make certain precautionary measures necessary, which are difficult to realize in the field. So the experiments were carried out in the laboratory, using the lowest possible amount of radio activity. This lowest possible amount however, must be sufficiently high to enable detection of a predator even after consumption of only one radioactive springtail. For determining the lowest possible amount, the effective decay of P^{32} in prey and predators had to be investigated.

The number of springtails predated can be estimated by the mortality of the springtails in the presence of the predators compared to their mortality in absence of the predators. A check on this estimate is possible by determining the amount of radioactivity in the predators, and calculating the corresponding number of springtails consumed. For this purpose also it is necessary to know the effective decay in prey and predators.

For the investigation of the effective decay in the predators the carabid beetle *Notiophilus biguttatus* F. and the pseudoscorpion *Neobisium muscorum* (Leach) were chosen. Both species are known springtail predators (Simon 1964, 1966). However, they differ in the manner of consuming the prey. *N. biguttatus* swallows its prey, whereas *N. muscorum* consumes its prey only partly after extra-intestinal digestion. Both species are numerous in the habitat of *O. cincta*, and *N. biguttatus* also in the wood where *T. minor* was collected.

2. Materials and methods

2.0. General remarks

All measurements on radioactivity are whole body counts, and were performed with the Nuclear-Chicago gas-flow detector, model 470, provided with a micromill-window, and operating as a Geiger-Müller counter at ± 1200 volt. This detector uses Q-gas (98.7% Helium and 1.3% Butane) at a pressure of 5 lbs/inch² (ca. 0.287 kg/cm²). In addition the Nuclear-Chicago sample chancer, model 1042, was used. This can contain maximally 50 planchettes, so that automatically 50 animals could be measured successively. Each measurement took one minute.

The isotope, in a watery solution of $Na_2H\ P^{32}O_4$, was mixed with the food of the springtails. This was for all experiments except one, similar to the food used by MÜLLER und BIERINGER (1971): 90 parts water, of which some was replaced by the watery solution of the radioactive combination, 5 parts maltagar, 5 parts milk and added to this 0.4% Nipagine (methyl-4-hydroxy benzoate) as fungicide. The springtails that had to be marked were kept together in a glass box (diam. 15 cm) with a plaster of Paris bottom (2 cm). After a starvation period the radioactive food was offered until the animals were marked sufficiently. Starvation was applied to minimize the variation in radioactivity among the individuals.

With the springtails marked in this way, the following experiments were carried out:

2.1. Experiments concerning the effective decay of P^{32} in the springtails and the predators

The springtails to be marked were fed for 4 days with the above mentioned food, to which radioactivity was added up to a concentration of 0.06 microcurie per milligram (μc/mg). The predators were made radioactive by feeding them with one or two radioactive springtails. After this marking and during the period of measurement the individuals were kept separately in small glass jars (diam. 2.5 cm) with a plaster of Paris bottom. The food of the springtails then consisted of greenalgae (*Pleurococcus* spec.). The predators were fed with non-radioactive springtails. Measurements were done for at least 4 weeks, with intervals of maximally one week.

2.2. Experiments concerning the effect of radioactivity upon the springtails

In one experiment mortality of three groups of individuals was compared for 4 weeks. One group was fed for 6 days with maltagar without radioactivity, one group with radioactivity in a concentration of 0.06 μc/mg, and the third group with radioactivity in a concentration of 0.12 μc/mg. After this treatment and during the period of observation the springtails were fed with greenalgae.

In a second experiment the possible difference in predation on radioactive individuals and non-radioactive individuals of the species *O. cincta* was evaluated. In this experiment 6 jars (diam. 15 cm) with a plaster of Paris bottom and covered with gauze, were supplied with a layer (± 7 cm) of pine needles. In each jar 20 radioactive springtails and 20 non-radioactive springtails were released, and also 5 individuals of the species *N. biguttatus* in each of 3 of the jars. In this case the springtails were starved for 2 days and then fed for 3 days with *Pleurococcus* spec. For the springtails to be marked, these greenalgae were soaked in a watery solution of the radioactive combination. This experiment lasted 8 days. Then the mortality of radioactive and non-radioactive springtails in each jar was determined.

2.3. Experiments to estimate the quantitative effect of the mortality factor predation

One experiment was done with *O. cincta*, and a second experiment with *T. minor*, which were collected in a pinewood and a birchwood respectively. In each experiment a piece of woodlandsoil, of 50 × 50 cm, was taken from the concerned wood to the laboratory. Its structure and contents remained unchanged. The plot was surrounded by metal plates and

placed in a large tray with a plaster of Paris bottom, which could be moistened. The plot was covered by gauze. In the experiment on *O. cincta* 200 radioactively marked springtails, previously collected in the same pinewood, were released in the plot. This experiment was performed in April. The population of *O. cincta* consists then only of adult hibernated individuals (Joosse 1969). The experiment on *T. minor* was performed in August. The population from which they were collected consisted mainly of young animals, born in spring and summer. Of this species 280 marked individuals were released in the plot. For both experiments the springtails were starved for 4 days, and then fed for 4 days with maltagar with radioactivity in a concentration of 0.06 μc/mg. Both experiments lasted eighteen days. Then the plots were carefully searched, all the animals of the litter layer collected and counted for radioactivity.

Mortality of the radioactive springtails in these plots was compared with mortality in woodlandsoil from which the predators were removed, and with their mortality living on *Pleurococcus* spec. in a glass box.

The radioactivity of the individual predators was added, and with the help of the data from the experiments on the effective decay of P^{32} in prey and predators, the corresponding number of radioactive springtails consumed was calculated.

All experiments described in this paper were performed at a temperature of 18—20 °C.

3. Results

3.1. Effective decay of P^{32} in *O. cincta*, *T. minor*, *N. biguttatus* and *N. muscorum*

It may be assumed that in the first period after labelling the animals, a rapid loss of radioactivity occurs, due to the excretion of the non-assimilated part of the food. This period was put at one week, because it certainly holds for the springtails that emptying of the gut occurs within a week (de With and Joosse 1971). During this period the radioactivity measured on a certain day (t_x) is expressed as a percentage of the radioactivity at t_0 (table 1). Radioactivity at t_0 for the springtails corresponds to their mean radioactivity in counts per minute (cpm) just after radioactive feeding, and for the predators to the mean radioactivity of the prey offered to them. The predators had consumed the prey animals after three days, so they were measured for the first time at t_3.

Loss of radioactivity after this first period is expressed by the mean effective half-life of P^{32} in days calculated from the exponential curves of decay that were determined for each individual.

With the two species of springtails and two species of predators 4 food chains can be constructed; using the data of table 1 and given the moment of predation, the amount of radioactivity present in each food chain at different moments can be calculated. This is shown, in percentages, in fig. 1.

Fig. 1 shows that when predation occurs the amount of radioactivity in the predator at t_{28}, ranges from 2.0 % to 12.7 %. This is dependent on the food chain concerned, and on

Table 1 Mean effective decay of P^{32} in the springtails and the two species of predators. For the mean effective half-lives 95 % confidence limits are recorded

Sample size	*O. cincta* 10	*T. minor* 12	*N. biguttatus* 12	*N. muscorum* 10
t_0	12,840	21,259	30,819	29,201
t_3			52.3	27.3
t_7	38.0	51.2		
t_{10}			15.4	12.9
mean eff. half-life	10.27	10.44	7.31	8.40
L_1	9.80	10.02	6.82	7.53
L_2	10.78	10.89	7.87	9.49

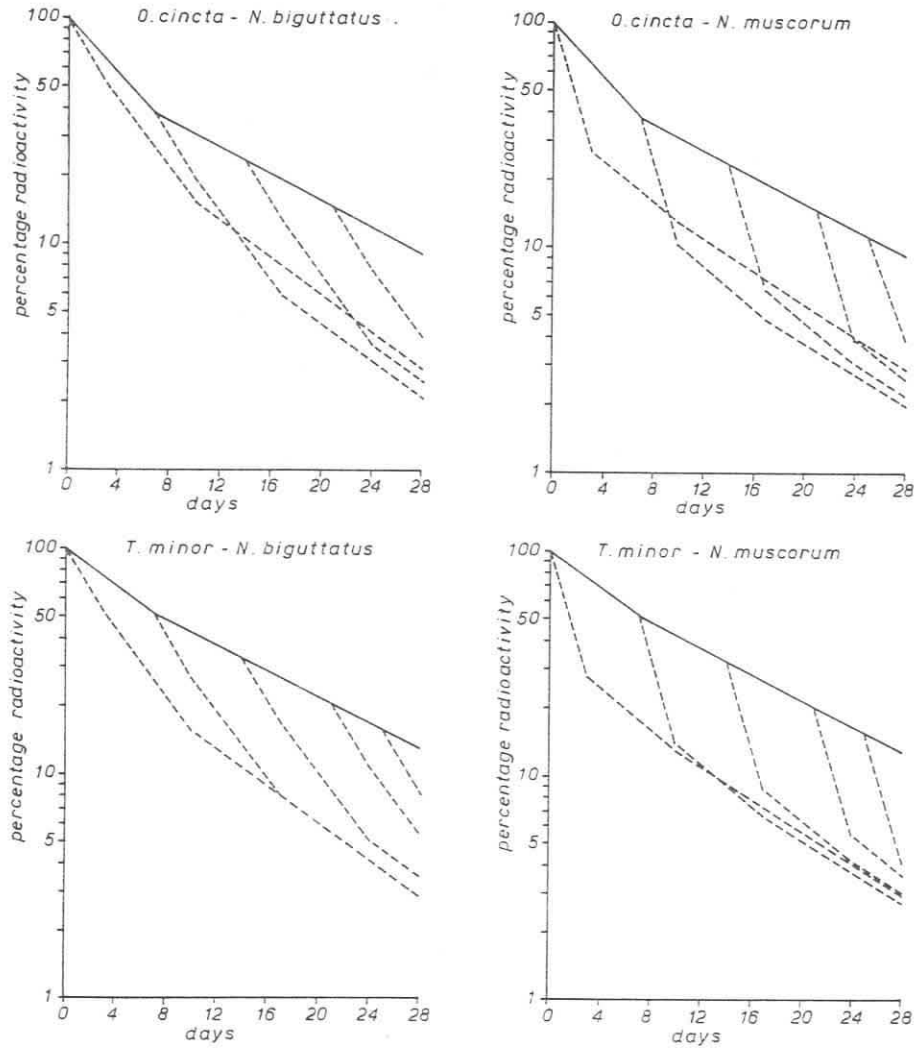

Fig. 1. Decay of radioactivity in prey (———) and in predators (·····), shown for four different foodchains. Decay via the predator is shown for different moments of predation.

the moment of predation: as effective decay is slower in the springtails than in the predators, it holds that the later predation takes place, the more radioactivity is left in the food chain. An exception to this rule occurs when the prey is predated immediately after the marking. In that case a relatively high amount of radioactivity is left in the food chain, because the first rapid loss of radioactivity from the springtails does not take place.

The minimum amount of radioactivity left from one springtail at the end of an experiment on predation must be detectable. When an amount of 50 cpm is taken as detectable (mean background 10 cpm) and the experiment will last 4 weeks, a prey animal must give at least 2500 cpm at the start of that experiment.

From different groups of springtails, each consisting of 20 individuals, fed with different concentrations of radioactivity and for a variable time, the radioactivity was measured. The results are presented in table 2.

Due to a high variation among individuals within each group the mean radioactivity has to be about 10.000 cpm, to ascertain that all individuals give more than 2.500 cpm. So it

Table 2 Radioactivity of the springtails (in counts/min.) fed with different concentrations of radioactivity and for a variable time

Concentration	Species	Duration		
		2 days	5 days	6 days
0.24 μc/mg	*O. cincta*	32,114	26,954	
	T. minor	38,953	41,248	
0.12 μc/mg	*O. cincta*		25,086	21,511
	T. minor		14,002	15,847
0.06 μc/mg	*O. cincta*		9,864	10,830
	T. minor		10,398	14,951

was decided to mark the springtails for the experiments on predation with radioactivity in a concentration of 0.06 μc/mg, and to stop the feeding when the springtails were sufficiently radioactive. This was established by a sample.

3.2. The effects of radioactivity upon the springtails

3.2.1. Mortality

Compared with man, many insects tolerate high doses of radioactive radiation (Bacq and Alexander 1961). This could hold also for springtails. Cavalloro and Delrio (1970) described the mortality of *Hypogastrura meridionalis*, which received doses of 10 krad and 20 krad, as being similar to the mortality of untreated animals. But Styron (1971) states that doses of beta radiation of 1885 rad upwards reduced survival of *Sinella curviseta*.

In the present study the mortality of non-radioactive individuals was compared with that of radioactive individuals (2 groups), with an average radioactivity as recorded in table 3. The total observation period was four weeks, being longer than the duration of the predation experiments.

It holds for both species that the differences in mortality of the three groups are not significant. Concerning *T. minor* it must be noted however, that all groups showed a high mortality.

Table 3 Mortality of radioactive and non-radioactive springtails after 4 weeks

Conc. of radioact. in the food	*O. cincta*			*T. minor*		
	nr.	Radioact.	Mortality (%)	nr.	Radioact.	Mortality (%)
nil	74	—	23	65	—	46
0.06 μc/mg	78	10,830 cpm	21	64	14,951 cpm	34
0.12 μc/mg	79	21,511 cpm	18	59	15,847 cpm	32

Table 4 Mortality after 8 days of radioactive and non-radioactive individuals of the species *O. cincta* in jars with and without predators

Total number of springtails	Jars without predators		Jars with predators	
	radioactive	non-radioactive	radioactive	non-radioactive
initial	60	60	60	60
final	58	58	42	40
died	2	2	18	20

Mean radioactivity of the marked springtails: 7580 cpm.

3.2.2. Predation chance

The results of the experiment carried out for comparing the predation chance of radioactive and non-radioactive springtails are shown in table 4.

From the data of table 4 a difference in predation chance between radioactive and non-radioactive springtails does not appear.

3.3. The effect of predation as a mortality factor

The litter structure and the relatively small size of the available individuals of *T. minor* made it much more difficult to recover *T. minor* in the woodland soil than *O. cincta* at the end of the experiments. So the percentages recorded in table 5 concerning *O. cincta* and the first group of *T. minor* may be read as survival percentages. The percentages on the woodland soil groups of *T. minor* however, ought to be read as percentages of recovery.

Table 5 Survival recovery of different groups of radioactive springtails

	Species/average initial radioactivity in cpm.					
	O. cincta/8487			*T. minor*/9824		
	initial number	final number	%	initial number	final number	%
Glass box — control	100	86	86.0	100	88	88.0
Woodland soil — control	100	89	89.0	100	70	70.0
Woodland soil — predation	200	118	59.0	280	119	42.5

The percentages recorded in table 5 are subject to chance fluctuations. With this in mind it can be concluded that of the experimental population of *O. cincta* 27—30 % was killed by predation, being the difference in survival between control groups and the experimental group. This is 66—73 % of the total mortality (41 %).

With respect to the experimental group of *T. minor* it was assumed that 18 %, which is the difference between the first and the second group, could not be recovered. Then the estimation of mortality due to predation is 27.5 %, which is 71 % of the total estimated mortality (39.5 %).

During the experiment on *T. minor* survival of 100 unmarked individuals of this species, living in a glass box on *Pleurococcus* spec., was also determined. The survival percentage of this group was 81 %, which is not significantly different from the 88 % survival of the first group of *T. minor* in table 5. This comparison was made because of the very high mortality of *T. minor* in the experiment described in section 3.2.1.

The quantitative effect of predation can also be calculated from the amount of radioactivity in the predators. Table 6 shows for both experiments the species, numbers and radioactivity of the predators.

In the experiment on *T. minor* some radioactive animals occurred (up to a total of 2500 cpm) with an unknown feeding habit.

As shown in fig. 1, the amount of radioactivity in the predators at the end of the experiment is a percentage of the initial radioactivity in the springtails predated, that can be calculated with the data of table 1. For that purpose it was assumed that each day an equal number of springtails was eaten, half by predators of the N. biguttatus-type and half by predators of the N. muscorum-type. Furthermore it had to be assumed that no secondary predation had occurred. With these percentages the number of springtails consumed can be calculated. The data are shown in table 7.

The predation on *O. cincta* calculated in this way shows a difference of 2.5—5.5 % with the percentage estimated from the survival values; with regard to *T. minor* this difference is

Table 6 Species, numbers and amount of radioactivity of radioactive predators in the experimental plots

		Experiment on *Orchesella cincta*			Experiment on *Tomocerus minor*		
Ordo	Fam.	No	Species	cpm.	No	Species	cpm.
Coleoptera	Carabidae	6	*Notiophilus biguttatus* F.	8460	2	*Notiophilus biguttatus* F.	3828
		4	*Amara plebeja* GYLL.	1774	5	*Amara brunnea* GYLL.	2182
		1	*Pterostychus vernalis* PANZ.	1668	5	*Trichocellus placides* GYLL.	8847
		1	*Amara aenea* GEER	201	6	*Trechus obtusus* ER.	5657
Coleoptera	Staphylinidae	3	*Philonthus splendens* F.	789	7	*Othius myrmecophilus* KIESW.	4646
		1	*Philonthus fuscipennis* MANNH.	978	1	*Tachyporus corticinus* GRAV.	980
		1	*Philonthus laminatus* CREUTZ	761	1	*Tachyporus marginellus* F.	395
		2	*Philonthus varius* GYLL.	791	1	*Tachyporus rufipes* DEG.	317
		1	*Philonthus sanguinolentus* GRAV.	140	11	larvae	9148
		1	*Xantholinus linearis* (OL.)	1066			
		1	*Baptolinus affinis* PAYK.	159			
		2	larvae	409			
Opilionida	Phalangiidae	1	*Milopus morio* (F.)	1138	1	*Milopus morio* (F.)	1668
Araneida	Theridiidae	8	*Enoplognatha thoracica* HAHN	4048			
	Salticidae	2	*Euophrys frontalis* WALCK.	2436			
	Lycosidae	1	*Trochosa* spec.	1487			
	Linyphiidae				3	*Centromerita bicolor* (BLACKWELL)	2730
					2	*Centromerus* spec.	2110
					2	*Diegmbium nigrum* (BLACKWELL)	753
					1	*Diplocephalus picinus* (BLACKWELL)	671
					1	*Tiso vagans* (BLACKWELL)	420
					1	*Agyneta* spec.	320
					3	unidentified	7362
Mesostigmata					7	unidentified	1846
Chelonethi	Neobisiidae	2	*Neobisium muscorum* (LEACH)	623			
Total radioactivity				26928			53880

Table 7 Predation of springtails calculated from the radioactivity in the predators

	Experiment	
	O. cincta	*T. minor*
Radioactivity in the predators	26,928 cpm	53,880 cpm
% of the initial prey-radioact.	6.5 %	8.2 %
Number of prey eaten	49	67
Predation as a percentage of the animals released	24.5 %	23.8 %

Table 8 Non-radioactive animals found in the plots at the end of the experiments

Experiment	Carabidae		Staphylinidae		Hydrophilidae	Opilionida	Araneida	Acari	Isopoda	Hymenoptera	Heteroptera	*O. cincta*	*T. minor*
	Larvae		Larvae										
O. cincta	2	1	6	3	—	—	14	> 100	—	—	—	126	—
T. minor	18	3	17	6	2	1	27	> 100	10	2	44	—	566

3.7 %. Calculation of predation in this way with the underlying assumptions will be discussed in section 4.

In view of an evaluation of the stability of predation as a mortality factor, the numbers of non-radioactive animals that were found in the litter layer of the plots at the end of the experiments, classified in main groups, are shown in table 8.

Most of the Carabid and Staphylinid beetles recorded in table 8 belonged to species, which also occurred as radioactive. The numbers of springtails recorded in table 8 are part of the springtails originally present in the plots; these numbers indicate that the density of the springtails was experimentally raised with about 94 % and 21 % in the experiments on *O. cincta* and *T. minor* respectively.

A comparison of table 6 and table 8 reveals for both experiments, especially for the O. cincta-experiment, that a large part of the animals of the litter layer feeds on the springtails. This will be discussed in section 4.

4. Discussion

It appeared from the present study that mortality of the two species of springtails *Orchesella cincta* and *Tomocerus minor* was very high in undisturbed woodland soil, compared with mortality in glass boxes and in woodland soil from which the predators had been removed.

To get the evidence that predation caused this raised mortality, all animals of the litter layer of the experimental plots were counted for radioactivity. The number of springtails that had to be consumed, to cause the amount of radioactivity in those animals with a predatory feeding habit was calculated.

Therefore it was assumed that each day the same number of springtails was predated. This assumption might be wrong: predation of the springtails possibly is a density dependant process. In that case more springtails would have been predated in the first half of the experiment compared to the second half. We should have calculated then with lower percentages as recorded in table 7, and the numbers of springtails predated should be greater than the numbers mentioned in table 7.

This applies also to the assumption about secondary predation: if this occurred, we had to calculate also with lower percentages.

The validity of the assumption that the data about effective decay given in table 1 are applicable to the animals in the woodland soil is doubtful. ODUM and GOLLEY (1961) described experiments which show that biological decay of zinc-65 is greater in the field than in the

laboratory. They stated that biological half-life is variable, especially for heterotherms, dependent on all kinds of ecological factors that influence the rate of metabolism.

In our experiments on predation (3.3.) the radioactivity of the remaining springtails indicates that effective decay in the woodland soil was probably greater than established in the experiments on effective decay (3.1.). In that case also, as with invalidity of the former assumptions, the numbers of springtails predated must have been greater than the numbers given in table 7, because we had to calculate with lower percentages.

From both experiments, percentages of mortality were obtained, that show the magnitude of the effect of predation on the numbers of the springtails. It must be noted however, that this effect is subject to variations in space and time. One of the major factors that determines the number of predated springtails is the density of the springtails. This factor changes considerably in space and time (Joosse 1969, 1970). Therefore experiments have been started to investigate the relation between predation and density of the prey, to enlarge our knowledge, obtained so far, about the effect of predation.

Another important and variable factor that causes fluctuations in the predation of the springtails is the density and feeding activity of the predators. Undoubtedly the variability of the effect of this factor on predation becomes less when predation is performed by more individuals and species. In fact, it was established in both experiments on predation that a great number of species and individuals prey on the springtails (table 6).

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6. Summary

Experiments were carried out in the laboratory to evaluate the importance of predation as a mortality factor for the two species of surface dwelling Collembola *Orchesella cincta* and *Tomocerus minor*. In each of these experiments a piece of woodland soil was brought into the laboratory, its structure and contents unchanged. A few hundred radioactively marked springtails of one species were released into this plot. The marked springtails and the plot originated from the same wood.

After 18 days the plots were searched, all animals of the litter layer collected and counted for radioactivity. From the survival of the marked springtails in the experimental plots, compared with survival in control plots, it appeared that with regard to both species considerable mortality by predation had occurred. This was estimated for both species on about 27 %, which was about 70 % of the total mortality.

A check on these percentages was obtained by calculating the springtails consumed from the amount of radioactivity in the predators. For this purpose experiments were carried out to investigate the effective decay of the used isotope (P^{32}) in the springtails and the predators. Using the data of these experiments, the calculations resulted in a mortality by predation of about 24 % for both species.

The number of species and individuals of predators that were radioactive at the end of the experiments, compared with the number of non-radioactive animals revealed that a large part of the soil animals feed on the springtails.

7. Literature

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